CYTOTOXIC PROPERTIES OF RHENIUM(I) TRICARBONYL COMPLEXES SUPPORTED BY *S,N,S'*-PINCER LIGANDS

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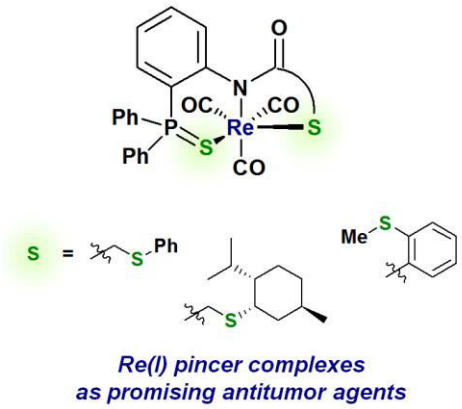
Abstract

Three Re(I) tricarbonyl complexes were readily obtained by the direct cyclometalation of non-classical pincer ligands based on functionalized amides which combine thiophosphoryl and thioether pendant arms. Realization of κ^3-*S,N,S'*-coordination in the resulting compounds was confirmed by multinuclear NMR and IR spectroscopy and, in the case of the phenylmercaptoacetyl derivative, also by X-ray crystallography. The complexes obtained were screened for cytotoxicity against human colon (HCT116), prostate (PC3), and breast (MCF7) cancer cell lines and exhibited moderate to good activity. The complexes bearing neomenthylmercaptoacetyl and methylmercaptobenzoyl coordination arms provided the same efficiency as a reference, cisplatin, in cancer cells, but were more selective towards human embryonic kidney cells HEK293, which were used as a representative of healthy cell lineages. Furthermore, the Re(I) pincer complex bearing a phenyl substituent at the thioether group exhibited promising activity against a doxorubicin-resistant subline of breast transformed cells HBL100.



Key words: rhenium, pincer complexes, functionalized amides, cytotoxicty.

The discovery of anticancer activity of cisplatin [1] and its further approval for clinical use marked a new era in medicinal chemistry and substantially shifted the focus to metal-based drugs. Nowadays, several platinum complexes are successfully used in clinical practice for treatment of different cancers, but intrinsic and acquired resistance as well as severe side effects caused by the platinum drugs lead to a constant need for creation of more effective and selective antitumor agents. To overcome the limitations associated with the platinum-based drugs, many efforts have been aimed to explore the potential of other transition metal compounds, including gold, ruthenium, titanium, zinc, and palladium derivatives [2]. More recently, there has been a growing interest in cytotoxic rhenium(I) complexes bearing a robust $Re(CO)_3$ core and various heterocyclic, organophosphorus, organoselenium, alkoxide or hydroxide ligands (for selected examples, see Fig. 1) [3]. The cytotoxic activities of these complexes were comparable or even exceeded that of cisplatin. Furthermore, some of the compounds exhibited also phototoxic effects. More importantly, recent studies revealed also high *in vivo* activity of several tricarbonylrhenium(I) complexes based on phenanthroline, diselenoether and β-carboline ligands, which mechanisms of action differ from that of traditional platinum-based drugs [4].

Most of the reported cytotoxic rhenium complexes contain mono- and bidentate ligands, and only a few examples are based on multidentate derivatives. The anticancer activity of cyclometallated Re(I) species remains almost unexplored. In our opinion, the application of multidentate ligands can provide controlled stability and better tunability of the potential drugs. Recently we have shown that tricarbonylrhenium(I) complexes

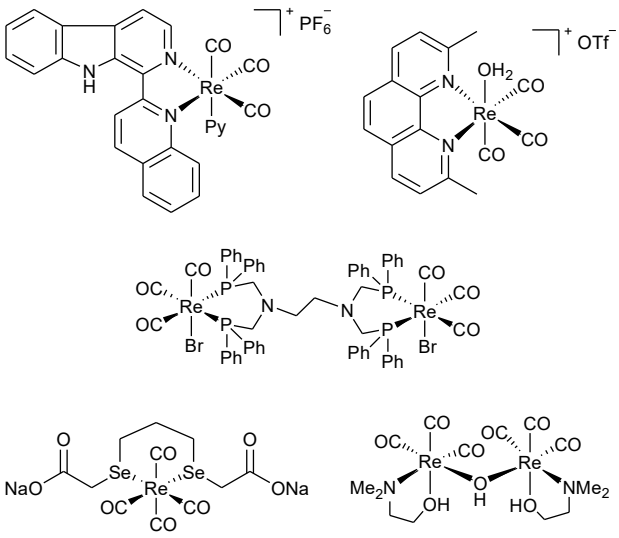
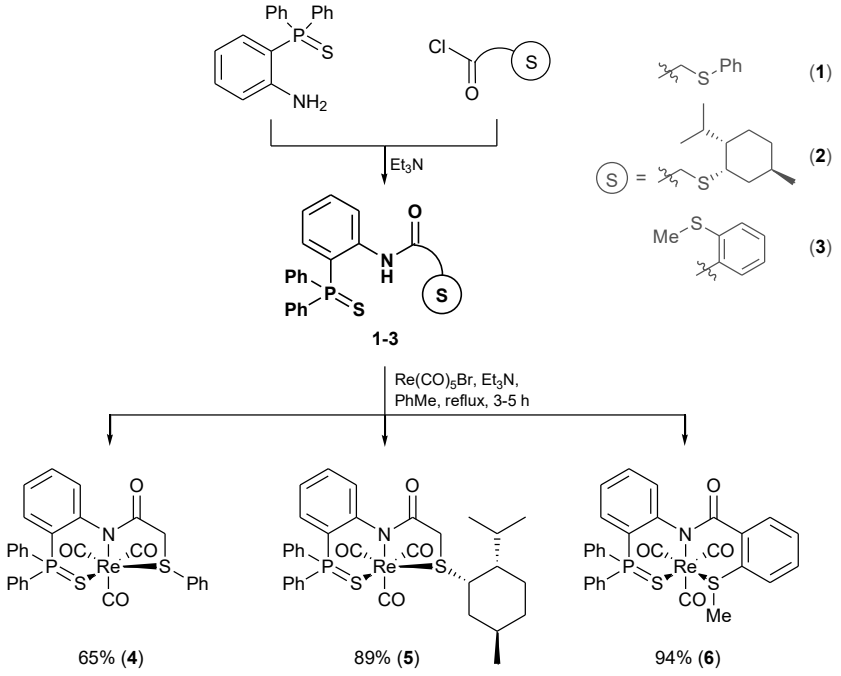


Figure 1. Selected examples of cytotoxic Re(I) complexes.

of functionalized amide ligands derived from 2-diphenylphosphorylaniline possess potent *in vitro* antitumor activity [5]. Furthermore, it was found that the pincer-type complex with a tridentate monoanionic ligand bearing, along with the P=O coordination arm, an ancillary thioether donor group exhibits higher tumor-growth inhibiting activity than its counterpart with a neutral bidentate coordination [5]. This result provides some credibility to our assumption, rendering further evaluation of anticancer potential of related Re(I) pincer complexes interesting. In this communication, we report on the synthesis and cytotoxicity studies of three new representatives of non-classical Re(I) pincer complexes based on functionalized amides combining thiophosphoryl and thioether coordination-active moieties.

The target ligands were obtained by the reactions of 2-diphenylthiophosphorylaniline with phenylmercaptoacetyl, neomenthylmercaptoacetyl, and 2-methylmercaptobenzoyl chlorides according to the published procedures (Scheme 1) [6, 7]. It should be noted that amides **1**–**3** have already been used as efficient ligands for Pd(II) ions and readily afforded Pd(II) pincer-type complexes, some of which demonstrated high catalytic activity in the Suzuki cross-coupling [6, 7] and remarkable cytotoxicity against several cancer cell lines [8]. The reactions of **1**–**3** with $Re(CO)_5Br$ upon refluxing in toluene in the presence of Et_3N smoothly provided Re(I) κ^3-*S,N,S'*-pincer complexes **4**–**6** (Scheme 1). Interestingly, complexes **4** and **5** featuring five- and six-membered fused metallocycles were readily formed and isolated in good yields (65–94%), independent from a substituent at the sulfur atom of the thioether group. However, we failed to obtain their counterpart with a flexible *S*-neomenthyl coordination arm having an ethylene unit [6]: even after prolonged heating of the reactants under similar conditions, the starting ligand remained intact. This is likely to be associated with an enhanced role of a steric factor on passing to the larger systems, *i.e.*, the bulkiness of the neomenthyl moiety hampered the production of 6,6-membered species. Nevertheless, changing the neomenthylmercapto-propanoyl arm for the less sterically hindered methylmercaptobenzoyl moiety readily afforded pincer complex **6**, which contains two six-membered fused metallocycles.

The complexes obtained were fully characterized by $^{31}P\{^1H\}$, ^1H, and $^{13}C\{^1H\}$ NMR as well as IR spectroscopy. Three distinct IR bands in the range of CO stretching vibrations (1888–2022 cm^{-1}) indicate the formation of *fac*-tricarbonyl species.[§] The absence of stretching and bending absorption bands characteristic for the free C(O)NH groups (in the range of 1500–1510 and 3200–3270 cm^{-1}) along with the concomitant lower-frequency displacement of the C=O bond stretching vibrations ($\Delta\nu$ = 66–89 cm^{-1}) evidence metalation of the central amide unit. The ^1H NMR spectra of **4**–**6** lack the downfield signals of the NH protons. The deprotonation and *N*-coordination of the amide units are also reflected in the expected changes in the $^{13}C\{^1H\}$ NMR spectra of the resulting complexes. The most important ones include strong downfield shifts of the signals of C=O and C1 (directly attached to the nitrogen atom) carbon nuclei: $\Delta\delta_C$ ~5.0–6.5 and ~14.6 ppm, respectively. Characteristic changes in the positions and multiplicities of the hydrogen and carbon resonances associated with the aliphatic substituents in the thioether moieties (methylene bridges, methyl or neomenthyl substituents) indirectly evidence the coordination of these ancillary donor groups. In turn, the lower-frequency shifts of the P=S bond stretches ($\Delta\nu$ = 27–35 cm^{-1}) unequivocally confirm coordination of the thiophosphoryl pendant arms. The latter is also supported by the downfield shifts of the phosphorus resonances in the NMR spectra of the complexes compared to those of the free ligands ($\Delta\delta_P$ reaches up to 1.64 ppm). Interestingly, the $^{31}P\{^1H\}$ NMR spectrum of complex **5** exhibits two singlets with close chemical shifts in *ca*. 1.6:1 ratio. These signals are likely to correspond to two diastereomers resulting from the complicated inversion of coordinated and noncoordinated electron pairs of the thioether sulfur atom bearing a chiral neomenthyl



Scheme 1. Synthesis of Re(I) tricarbonyl complexes supported by *S,N,S'*-pincer ligands.

substituent. Of note is the preferential formation of one of the isomeric forms, which is presumably stipulated by a facial arrangement of the tridentate ligand. Two sets of signals are observed also in the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of this compound. Whereas the ^{1}H NMR spectrum contains complicated patterns of overlapping signals (especially in the region of aromatic proton signals), most of the carbon resonances of both minor and major isomer can be readily assigned (see Experimental). It should be noted that the presence of a bulky neomenthyl substituent leads to stabilization of this system at room temperature, while both of its counterparts **4** and **6** undergo dynamic transformations, which involve mainly the thioether pendant arms. Thus, the ^{1}H NMR spectrum of complex **6** registered at room temperature contain broadened, unresolved signals of the C_6H_4SMe protons; its $^{13}C\{^{1}H\}$ NMR spectrum display only a part of the expected carbon resonances. Unfortunately, we failed to obtain the high-quality NMR spectra of this compound at low temperatures (due to its low solubility). However, in the case of complex **4**, cooling to –15 °C resulted in the resolution of almost all the signals of the thioether pendant arm both for the hydrogen and carbon nuclei (see Figs. S1–S3 in Electronic supplementary information (ESI)). For example, the protons of the methylene unit gave rise to a well-resolved AB quartet, which is consistent with the prochiral nature of this group upon coordination of the thioether moiety by Re(I) ions (Fig. S2 in ESI). Furthermore, 2D NMR spectroscopy (COSY, HSQC, and HMBC pulse sequences) at the reduced temperature allowed us to fully assign the signals of complex **4** (see Figs. S4–S10 in ESI and Experimental).

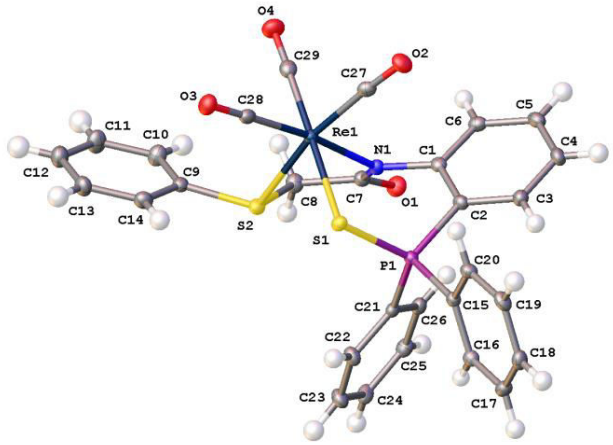


Figure 2. General view of complex **4** in representation of atoms *via* thermal ellipsoids at 50% probability level. Selected bond distances (Å) and angles (deg): Re1–C27 1.924(2), Re1–C28 1.917(2), Re1–C29 1.924(2), Re1–S1 2.5320(6), Re1–S2 2.4835(6), Re1–N1 2.1914(19), P1–S1 2.0100(8), N1–C7 1.343(3), O1–C7 1.237(3), C27–Re1–C28 91.34(10), C28–Re1–C29 88.54(10), C27–Re1–C29 92.18(10), S1–Re1–N1 88.69(5), S2–Re1–N1 74.04(5), S1–Re1–S2 88.882(19), C27–Re1–S2 168.69(7), C28–Re1–N1 173.22(8), C29–Re1–S1 178.31(7).

We were also successful in authentication of compound **4** by single-crystal XRD (Fig. 2). This complex exhibits the expected distorted octahedral geometry for the Re(I) center and the facial arrangement of the deprotonated tridentate amide ligand. The Re–C bond distances are consistent with those found in the related complexes (see, for example, Ref. [5]). The Re1–S2 (2.4835(6) Å) distance is slightly shorter than Re1–S1 (2.5320(6) Å) due to different natures of the sulfur donors. The *trans* C–Re–X angles (X = S, N) range within 168.69(7)–178.31(7)°, whereas the values of *cis* angles C–Re–C and X–Re–Y (X, Y = S, N) fall within 74.04(5)–92.18(10)°, which indicates major deviations from the ideal octahedral geometry, which result from the conjugation of five- and six-membered metal-containing rings.

The cytotoxic activities of the complexes obtained were tested against several cancer cell lines, including human colon (HCT116), breast (MCF7), and prostate (PC3) cancer. The results are presented in Table 1 as the concentrations required for 50% inhibition of cell growth. As can be seen, complex **4** demonstrated an appreciable level of cytotoxicity only in the case of HCT116 cancer lineage, while in the breast and prostate cancer cells it was only moderately active. At the same time, its counterparts **5** and **6** produced remarkable cytotoxic effects on all the malignant cells explored. The values of IC_{50} for these complexes ranged within 17.0–36.0 μM and were comparable to those of a reference—cisplatin. Furthermore, they were less toxic towards normal human embryonic kidney cells HEK293 than the clinically used platinum-based drug. However, both complexes **5** and **6** were almost inactive in transformed breast cells HBL100 and their doxorubicin-resistant subline HBL100/Dox, while compound **4** displayed promising antiproliferative activity: the corresponding values of IC_{50} were almost equal and composed 28.0 ± 6.0 and 32.0 ± 2.0 μM, respectively. This offers opportunities for the development of Re(I) tricarbonyl complexes supported by *S,N,S'*-pincer ligands that will be able to overcome drug resistance. Further investigations on modification of functionalized amide ligand framework present an interesting line of research for the creation of new rhenium-based antitumor agents.

Experimental

General remarks

All manipulations were carried out without taking precautions to exclude air and moisture. Dichloromethane was distilled from P_2O_5. Ligands **1**, **2** [6], and **3** [7] were synthesized according to the published procedures. All other chemicals and solvents were used as purchased.

Table 1. Cytotoxic activities of rhenium(I) complexes **4**–**6** (IC_{50}, μM)

Entry	Compound	Cancer cell lines			Healthy cell line
		HCT116	MCF7	PC3	HEK293
1	**4**	25.5 ± 2.5	50.0 ± 8.0	54.0 ± 8.5	70.0 ± 16.0
2	**5**	17.5 ± 3.5	32.0 ± 5.5	23.0 ± 2.5	32.0 ± 2.5
3	**6**	17.0 ± 5.0	24.5 ± 4.5	36.0 ± 10.0	34.0 ± 8.5
4	cisplatin	18.0 ± 2.0	25.0 ± 4.0	16.0 ± 3.0	12.5 ± 1.5

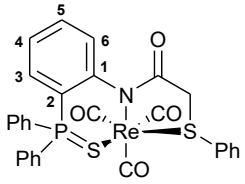
The NMR spectra were recorded on Bruker Avance 400 and Avance 500 spectrometers, and the chemical shifts (δ) were referenced internally by the residual (^{1}H) or deuterated (^{13}C) solvent signals relative to tetramethylsilane or externally to H_3PO_4 (^{31}P). The $^{13}C\{^1H\}$ NMR spectra were registered using the *J*MODECHO mode; the signals for the C nuclei bearing odd and even numbers of protons have opposite polarities. The assignment of the NMR spectra of complexes **4** and **6** was carried out based on the ^1H–^1H-COSY, HSQC, and HMBC experiments. Along with the previously reported data for the related compounds [6, 7], the results obtained were used to assign the NMR spectra of complex **5**.

The IR spectra were recorded on a Nicolet Magna-IR750 FT spectrometer (resolution 2 cm^{-1}, 128 scans). The assignment of absorption bands in the IR spectra was made according to Ref. [9]. Column chromatography was carried out using Macherey-Nagel silica gel 60 (MN Kieselgel 60, 70–230 mesh). Melting points were determined with an MPA 120 EZ-Melt automated melting point apparatus (Stanford Research Systems).

Syntheses

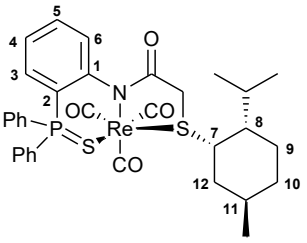
General procedure for the synthesis of [κ^3-*S,N,S'*-(L)Re(I)(CO)$_3$] complexes 4–6. A stirred mixture of the corresponding ligand (0.251 mmol), $Re(CO)_5Br$ (102 mg, 0.251 mmol), Et_3N (35 μL, 0.251 mmol), and toluene (10 mL) was refluxed for 3 (in the case of ligands **1**, **2**) or 5 h (in the case of ligand **3**). After cooling to room temperature, the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel (eluent: CH_2Cl_2 (**1**), CH_2Cl_2–MeOH (50:1) (**2**), CH_2Cl_2–EtOH (20:1) (**3**)) to give the target complexes as light-beige (**4**, **5**) or white (**6**) crystalline solids.

Complex 4.



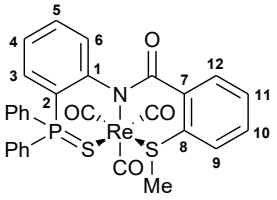
Yield: 119 mg (65%). Mp: >253 °C (dec.). $^{31}P\{^1H\}$ NMR (202.47 MHz, $CDCl_3$, 258 K): δ 41.23 ppm. ^{1}H NMR (500.13 MHz, $CDCl_3$, 258 K): δ 3.63 and 3.70 (ABq, 2H, CH_2, J_{AB} = 14.1 Hz), 6.85 (dd, 1H, H(C3), $^3J_{HP}$ = 14.3 Hz, $^3J_{HH}$ = 7.7 Hz), 7.12–7.15 (m, 1H, H(C4)), 7.43–7.51 (m, 4H, H(C6) + 3H$_{Ar}$ in SPh), 7.55–7.61 (m, 6H, *m*-H in P(S)Ph + 2H$_{Ar}$ in SPh), 7.64–7.73 (m, 5H, H(C5) + *o*-H in P(S)Ph + *p*-H in P(S)Ph$_2$), 7.76–7.80 (m, 2H, *o*-H in P(S)Ph) ppm. $^{13}C\{^1H\}$ NMR (125.76 MHz, $CDCl_3$, 258 K): δ 44.60 (s, CH_2), 123.09 (d, C2, $^1J_{CP}$ = 86.4 Hz), 124.58 (d, C4, $^3J_{CP}$ = 13.2 Hz), 126.46 (d, *ipso*-C in P(S)Ph, $^1J_{CP}$ = 86.4 Hz), 127.22 (d, *ipso*-C in P(S)Ph, $^1J_{CP}$ = 87.9 Hz), 127.49 (d, C6, $^3J_{CP}$ = 7.7 Hz), 128.55 (d, *m*-C in P(S)Ph, $^3J_{CP}$ = 13.4 Hz), 129.25 (d, *m*-C in P(S)Ph, $^3J_{CP}$ = 12.4 Hz), 129.92 (s, *p*-C in SPh), 130.03 and 130.24 (both s, *o*-C and *m*-C in SPh), 131.78 (s, *ipso*-C in SPh), 132.58 (d, C3, $^2J_{CP}$ = 9.5 Hz), 132.78 (d, *o*-C in P(S)Ph, $^2J_{CP}$ = 11.6 Hz), 133.23–133.29 (m, overlapping signals of *p*-C in P(S)Ph$_2$), 133.51 (d, *o*-C in P(S)Ph, $^2J_{CP}$ = 10.5 Hz), 134.84 (d, C5, $^4J_{CP}$ = 2.5 Hz), 155.18 (d, C1, $^2J_{CP}$ = 3.6 Hz), 173.27 (s, C=O), 191.01 and 191.04 (both s, 2CO), 194.64 (s, CO) ppm. IR (KBr, ν/cm^{-1}): 415(w), 480(w), 528(m), 600(m) (νP=S), 620(w), 641(w), 690(m), 704(m), 720(w), 742(m), 750(sh, w), 760(w), 982(w), 999(w), 1103(m), 1131(w), 1159(w), 1187(w), 1265(w), 1343(m), 1404(w), 1438(m), 1462(m), 1481(w), 1565(w), 1578(m), 1617(s) (νC=O), 1888(vs) (CO), 1919(vs) (CO), 2022(vs) (CO), 3054(w). Anal. Calcd for $C_{29}H_{21}NO_4PReS_2$: C, 47.79; H, 2.90; N, 1.92. Found: C, 47.82; H, 2.80; N, 1.84%.

Complex 5.



Yield: 177 mg (89%). Mp: >220 °C (dec.). $^{31}P\{^1H\}$ NMR (161.98 MHz, $CDCl_3$, major isomer (M) 62%, minor isomer (m) 38%): δ 41.24 (m), 41.45 (M) ppm. ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 0.89–1.91 (m, 17H, H$_{Alk}$ (M) + 17 H, H$_{Alk}$ (m)), 2.27–2.39 (m, 1H, H$_{Alk}$ (M) + 1H, H$_{Alk}$ (m)), 3.19–3.22 (m, 1H, CH_2S (m)), 3.34–3.38 (m, 1H, CH_2S (M) + 1H, CH_2S (m)), 3.57–3.67 (m, 1H, CH_2S (M) + 1H, H(C7) (M) + 1H, H(C7) (m)), 6.77–6.84 (m, 1H, H$_{Ar}$ (M) + 1H, H$_{Ar}$ (m)), 7.08–7.12 (m, 1H, H$_{Ar}$ (M) + 1H, H$_{Ar}$ (m)), 7.39–7.77 (m, 12H, H$_{Ar}$ (M) + 12H, H$_{Ar}$ (m)) ppm. $^{13}C\{^1H\}$ (100.61 MHz, $CDCl_3$): δ 20.65 (s, Me (M)), 20.80 (s, Me (m)), 21.68 (s, Me (M)), 21.72 (s, Me (M)), 21.85 (s, Me (m)), 22.10 (s, Me (m)), 24.60 (s, C9 (m)), 24.66 (s, C9 (M)), 27.54 (s, CH (m)), 27.63 (s, CH (M)), 29.43 (s, CH (M)), 29.65 (s, CH (m)), 34.51 (s, CH_2S (m)), 35.02 (s, CH_2S (M)), 38.79 (s, CH_2 (m)), 40.42 (s, CH_2 (M)), 41.48 (s, CH_2 (M)), 45.13 (s, CH_2 (m)), 49.63 (s, CH (M + m)), 52.56 (s, CH (M)), 53.25 (s, CH (m)), 122.64 (d, C2 (m), $^1J_{CP}$ = 86.4 Hz), 122.89 (d, C2 (M), $^1J_{CP}$ = 86.8 Hz), 124.17 (d, C4 (M + m), $^3J_{CP}$ = 13.2 Hz), 126.87 (d, *ipso*-C in P(S)Ph (M), $^1J_{CP}$ = 86.3 Hz), 126.98 (d, *ipso*-C in P(S)Ph (m), $^1J_{CP}$ = 86.2 Hz), 127.74 (d, C6 (m), $^3J_{CP}$ = 7.7 Hz), 127.76 (d, *ipso*-C in P(S)Ph (M), $^1J_{CP}$ = 88.5 Hz), 127.89 (d, C6 (M), $^3J_{CP}$ = 7.9 Hz), 128.05 (d, *m*-C in P(S)Ph (M), $^3J_{CP}$ = 13.7 Hz), 128.09 (d, *m*-C in P(S)Ph (m), $^3J_{CP}$ = 13.8 Hz), 128.90 (d, *m*-C in P(S)Ph (M + m), $^3J_{CP}$ = 12.5 Hz), 132.06 (d, C3 (M), $^2J_{CP}$ = 9.5 Hz), 132.11 (d, C3 (m), $^2J_{CP}$ = 9.5 Hz), 132.53 (d, *p*-C in P(S)Ph$_2$ (m), $^4J_{CP}$ = 3.3 Hz), 132.66 (d, *o*-C in P(S)Ph (m), $^2J_{CP}$ = 11.3 Hz), 132.71 (d, *o*-C in P(S)Ph (M), $^2J_{CP}$ = 11.7 Hz), 132.85 (d, *p*-C in P(S)Ph$_2$ (M), $^4J_{CP}$ = 3.2 Hz), 133.34 (d, *o*-C in P(S)Ph (M + m), $^2J_{CP}$ = 10.4 Hz), 134.32 (d, C5 (M), $^4J_{CP}$ = 2.3 Hz), 134.40 (d, C5 (m), $^4J_{CP}$ = 2.2 Hz), 155.28 (d, C1 (M + m), $^2J_{CP}$ = 3.8 Hz), 173.63 (s, C=O (M)), 173.75 (s, C=O (m)), 190.58 (s, CO (M)), 190.63 (s, CO (m)), 190.71 (s, CO (M)), 190.87 (s, CO (m)), 194.50 (s, CO (m)), 194.56 (s, CO (M)) ppm (the signal of *ipso*-C nucleus in P(S)Ph moiety of the minor isomer was not resolved). IR (KBr, ν/cm^{-1}): 526(m), 601(m) (νP=S), 620(w), 641(w), 691(m), 705(m), 720(w), 750(w), 998(w), 1103(m), 1130(w), 1187(w), 1265(w), 1349(m), 1438(m), 1463(m), 1580(m), 1622(s) (νC=O), 1893(vs) (CO), 1916(vs) (CO), 2019(vs) (CO), 2872(w), 2924(m), 2954(m), 3058(w). Anal. Calcd for $C_{33}H_{35}NO_4PReS_2$: C, 50.11; H, 4.46; N, 1.77. Found: C, 50.19; H, 4.37; N, 1.69%.

Complex 6.



Yield: 172 mg (94%). Mp: >238 °C (dec.). $^{31}P\{^1H\}$ NMR (161.98 MHz, $CDCl_3$): δ 41.55 ppm. ^1H NMR (500.13 MHz, $CDCl_3$): δ 2.70 (br. s, 3H, SMe), 6.72 (ddd, 1H, H(C3), $^3J_{HP}$ = 15.1 Hz, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.5 Hz), 7.02–7.07 (m, 2H, H(C4) + 1H_{Ar} in C_6H_4SMe), 7.30–7.35 (m, 2H, 1H_{Ar} in C_6H_4SMe + 1H_{Ar}), 7.40–7.46 (m, 3H, H_{Ar}), 7.49 (dd, 1H, H(C6), $^3J_{HH}$ = 7.8 Hz, $^4J_{HP}$ = 5.9 Hz), 7.56–7.60 (m, 2H, *m*-H in P(S)Ph), 7.66–7.73 (m, 4H, H(C5) + 3H_{Ar}), 7.83 (ddd, 2H, *o*-H in P(S)Ph_2, $^3J_{HP}$ = 14.8 Hz, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.6 Hz), 8.15 (br. d, 1H, H_{Ar} in C_6H_4SMe, $^3J_{HH}$ = 7.4 Hz) ppm. $^{13}C\{^1H\}$ NMR (125.76 MHz, $CDCl_3$): δ 22.49 (s, SMe), 123.68 (d, C4, $^3J_{CP}$ = 13.2 Hz), 127.57 (d, *ipso*-C in P(S)Ph, $^1J_{CP}$ = 86.6 Hz), 127.76 (d, *ipso*-C in P(S)Ph, $^1J_{CP}$ = 87.8 Hz), 128.50 (d, *m*-C in P(S)Ph, $^3J_{CP}$ = 14.1 Hz), 129.06 (d, *m*-C in P(S)Ph, $^3J_{CP}$ = 12.7 Hz), 129.21 (d, C6, $^3J_{CP}$ = 8.6 Hz), 129.33 (br. s, unresolved signal of C_{Ar} in C_6H_4SMe), 131.76 (d, C3, $^2J_{CP}$ = 9.7 Hz), 132.87 (d, *p*-C in P(S)Ph, $^4J_{CP}$ = 3.4 Hz), 132.95 (d, *o*-C in P(S)Ph, $^2J_{CP}$ = 11.8 Hz), 133.09 (d, *p*-C in P(S)Ph, $^4J_{CP}$ = 2.7 Hz), 133.51 (br. s, C_{Ar} in C_6H_4SMe), 133.75 (d, *o*-C in P(S)Ph, $^2J_{CP}$ = 10.5 Hz), 134.40 (d, C5, $^4J_{CP}$ = 2.5 Hz), 170.98 (s, C=O), 189.62 (s, CO), 190.91 (s, CO), 193.34 (s, CO) (the signals of the other carbon nuclei were not observed) ppm. IR (KBr, ν/cm^{-1}): 504(w), 523(w), 592(w), 604(m) (νP=S), 620(w), 640(w), 690(m), 703(w), 717(w), 748(m), 1104(m), 1264(w), 1328(s), 1437(m), 1465(w), 1559(m), 1578(m), 1595(s) (νC=O), 1913(vs, br) (CO), 2022(vs) (CO), 2976(vw), 3057(vw). Anal. Calcd for $C_{29}H_{21}NO_4PReS_2$: C, 47.79; H, 2.90; N, 1.92. Found: C, 48.06; H, 3.16; N, 1.84%.

X-ray crystallography

Single crystals of **4** (obtained by slow crystallization from CH_2Cl_2–hexane; $C_{29}H_{21}NO_4PReS_2$, M = 728.81) are triclinic, space group P-1, at 120 K: a = 9.4678(4), b = 10.2957(4), c = 13.8511(6) Å, α = 84.4940(10)°, β = 79.4180(10)°, γ = 85.2510(10)°, V = 1318.15(10) Å^3, Z = 2 (Z' = 1), d_{calc} = 1.836 g cm^{-3}, μ(MoKα) = 48.66 cm^{-1}, F(000) = 712. Intensities of 17975 reflections were measured with a Bruker APEX2 DUO CCD diffractometer (λ(MoKα) = 0.71073 Å, ω-scans, 2θ < 58°), and 6998 independent reflections (R_{int} = 0.0263) were used in further refinement. Using Olex2 [10], the structure was solved with the ShelXT [11] structure solution program *via* Intrinsic Phasing and refined with the olex2.refine [12] refinement package using Gauss-Newton minimization. Positions of the hydrogen atoms were calculated and then were refined in the isotropic approximation within the riding model. The refinement converged to wR2 = 0.0462 and GOF = 1.027 for all the independent reflections (R1 = 0.0195 was calculated against F for 6556 observed reflections with $I > 2\sigma(I)$). CCDC 1974483 contains the supplementary crystallographic information for **4**. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Cytotoxicity studies

The cytotoxic properties of Re(I) complexes **4**–**6** were studied against human colon (HCT116), breast (MCF7), and prostate (PC3) cancer cell lines as well as healthy human embryonic kidney cells HEK293. Additional experiments were carried out on transformed breast cells HBL100 and a doxorubicin-resistant subline HBL100/Dox. RPMI-1640 and DMEM media were obtained from Gibco. Fetal bovine serum (FBS) was purchased from HyClone. Cells were cultured in RPMI-1640 (in the case of PC3 and HBL100) or DMEM (in the other cases) media supplemented with 10% FBS and 50 μg/mL gentamicin in a humidified incubator with 5% CO_2 atmosphere. The effect of the compounds on cell viability was evaluated by the standard MTT assay (ICN Biomedicals, Germany). Cells were seeded in triplicate at a cell density of 5×10^3/well in 96-well plates in 100 μL complete medium and preincubated for 24 h. The tested compounds were initially dissolved in DMSO. Then the compounds at various concentrations were added to the media. The well plates were incubated for 48 h followed by addition of MTT solution (Sigma) (20 μL, 5 mg/mL). The cells were incubated at 37 °C for further 3 h; then the culture medium was removed, and formazan crystals were dissolved in DMSO (70 μL). The absorbance of the resulting solutions was measured on a multi-well plate reader (Multiskan FC, Thermo scientific) at 540 nm to determine the percentage of surviving cells. The reported values of IC_{50} are the averages of three independent experiments (Table 1).

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Electronic supplementary information

Electronic supplementary information (ESI) available online: variable-temperature ^1H and $^{13}C\{^1H\}$ NMR spectra for complex **4**; ^1H, $^{13}C\{^1H\}$, ^1H–^1H COSY, HSQC, and HMBC NMR spectra for compound **4** at –15 °C. For ESI, see DOI: 10.32931/io1923a.

References and notes

§ In the case of complex **6**, two ν(CO) stretches overlap, resulting in a single broadened band. Nevertheless, the $^{13}C\{^1H\}$ NMR spectrum of this compound exhibits three singlet signals

in the range of 189.6–193.3 ppm, which confirms the facial arrangement of three carbonyl ligands.

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